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Morphological Evidence of Hybridization between *Aconitum japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* (Ranunculaceae)

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Examination of pedicel pubescence and morphological analyses of *Aconitum* populations at Mt. Chiri area in Korea revealed that they were derived from hybridization between *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense*. The two taxa were clearly separated by pedicel pubescence and a number of other vegetative and floral characters measured, while the putative hybrid populations occupied an intermediate position. In addition, the absence of "typical" forms of both *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* in the hybrid populations as well as in Mt. Chiri area suggests that repeated introgression has probably occurred in these populations.

Introduction

Aconitum L. (Ranunculaceae) is a taxonomically difficult and highly variable genus consisting of about 300 species (Kadota 1987). The species of *Aconitum* are annual, biennial or perennial herbs, and widely distributed in cold temperate to temperate regions of the Northern Hemisphere including Asia, North America, and Europe, with the greatest concentration of species in eastern Asia (Hardin 1964, Tamura 1966a). The genus is usually defined within the Ranunculaceae by zygomorphic flowers and spurred, long-stipitate, nectariferous petals (Tamura 1966b), and has been divided into three subgenera mainly on the basis of habit and shape of underground parts; these include subg. Aconitum with biennial tubers, subg. Lycoctonum Tourn. with perennial rhizomes, and subg. Gymnaconitum (Stapf) Rapaics with annual roots (Wang 1979, Kadota 1987).

Aconitum japonicum Thunb. ex Murray belongs to the subg. Aconitum sect. Euchylodea Rchb. ser. Japonica (Nakai) Kadota, and differs from other species in the series in having pedicels moderately to densely pubescent with curved hairs. Kadota (1987), in his revision of *Aconitum* subg. Aconitum of East Asia, recognized five subspecies of *A. japonicum* based on the differences in leaf shape, helmet (upper sepal) shape, and habit of branches; these include subsp. *japonicum*, *ibukiense* (Nakai) Kadota, *subcuneatum* (Nakai) Kadota, *maritimum* (Nakai ex Tamura & Namba) Kadota, and *napiforme* (Lév. & Vaniot) Kadota. *Aconitum japonicum* subsp. *napiforme*, originally described as a distinct species by Léveillé and Vaniot (in Léveillé 1908) based on the specimen from Cheju Island in Korea, can be distinguished from other subspecies by its ternate leaves, hooded helmets with long projecting beaks, slender and relatively short nectary spurs, and glabrous stamens (Kadota 1987).

Unlike other subspecies of *A. japonicum*, which are restricted to Japan, subsp. *napiforme* is distributed from southern Japan through Korea to northeastern China (Kadota 1987). In Korea, it is mainly distributed in southern part including Mt. Chiri and Cheju Island, and usually occupies in shady, moist places with rich soils under the mixed deciduous forests along the mountain ridges and slopes.

During a comprehensive systematic study on the subg. Aconitum in Korea, natural populations containing putative hybrids involving *A. japonicum* subsp. *napiforme* and *A. jaluense* Komarov subsp. *jaluense* were found along the ridges of Mt. Chiri in Korea. These populations contain individuals having both micropapillate curved hairs and varying densities of smooth-surfaced unicellular spreading glandular hairs on pedicels. In addition, individuals of these populations somewhat resemble *A. jaluense* subsp. *jaluense* in leaf shape, and their chromosome number is 2n=32 as in *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* (Park and Oh unpubl. data). Recent studies suggested that interspecific hybridization is relatively common in subg. Aconitum, and many intermediate forms possibly derived from interspecific hybridization have been reported (Hardin 1964, Kadota 1981, 1987).

The main objective of this paper is to document hybridization and possible introgression between *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* using morphological analyses of individuals of suspected hybrids and parental types.

Materials and Methods

Four natural populations that included suspected hybrids were located along the ridges (alt. ca. 1,200–1,700 m) of Mt. Chiri in southern part of Korea (Fig. 1). From these populations, a total of 82 individuals representing different morphological forms was collected. For three of these populations, 22 to 27 individuals per population were collected, but one at Simwon region was relatively small in size and eight individuals were sampled. In addition, 50 individuals of "pure" *A. japonicum* subsp. *napiforme* collected from Cheju Island, which is the type locality of the taxon, and 47 individuals of "pure" *A. jaluense* subsp. *jaluense* from different localities in South Korea were examined for morphological analyses. For these taxa, specimens were selected to represent morphological variability of each taxon. All vouchers are at SNU, and a list of specimens used for this study is available from the first author upon request.

From these specimens, 20 vegetative characters, seven of which were derived ratios, were measured for morphological analyses (Table 1, characters 1–20; Fig. 2A). For leaf characters, a large, fully mature leaf in each individual was used for measurements. Then a subset of 93 individuals, including 29 of *A. japonicum* subsp. *napiforme*, 40 of *A. jaluense* subsp. *jaluense*, and 24 of the putative hybrid populations, was measured for four floral characters (Table 1, characters 21–24; Fig. 2B); many individuals were excluded from the measurements due to the lack of appropriate flowers. For the putative hybrid population at Simwon region, only one individual was available for measurements of floral characters.

To analyze better the morphological distinctness of *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* and the position of the putative hybrids in relation to the parental taxa, a data set derived from the measurements of 20 vegetative characters (characters 1–20; Table 1) was subjected to principal components analysis. The floral characters were not included in the analysis, because many individuals lacked appropriate flowers for measurements. The analysis was performed on a correlation matrix and carried out using Statistical Analysis System (SAS Institute 1990; Release 6.04) on an IBM PC. Data matrix used for the analysis is available

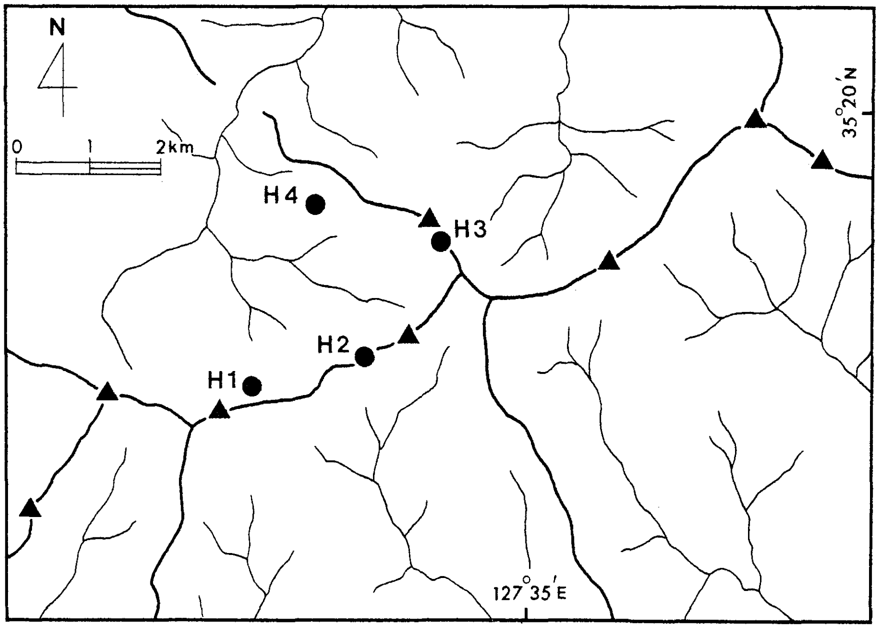

Fig. 1. Locations for four populations derived from hybridization between *Aconitum japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* in Mt. Chiri area. H1: Nogodan, alt. 1,500 m. H2: Imgeolryung, alt. 1,400 m. H3: Banyabong, alt. 1,700 m. H4: Simwon, alt. 1,200 m.

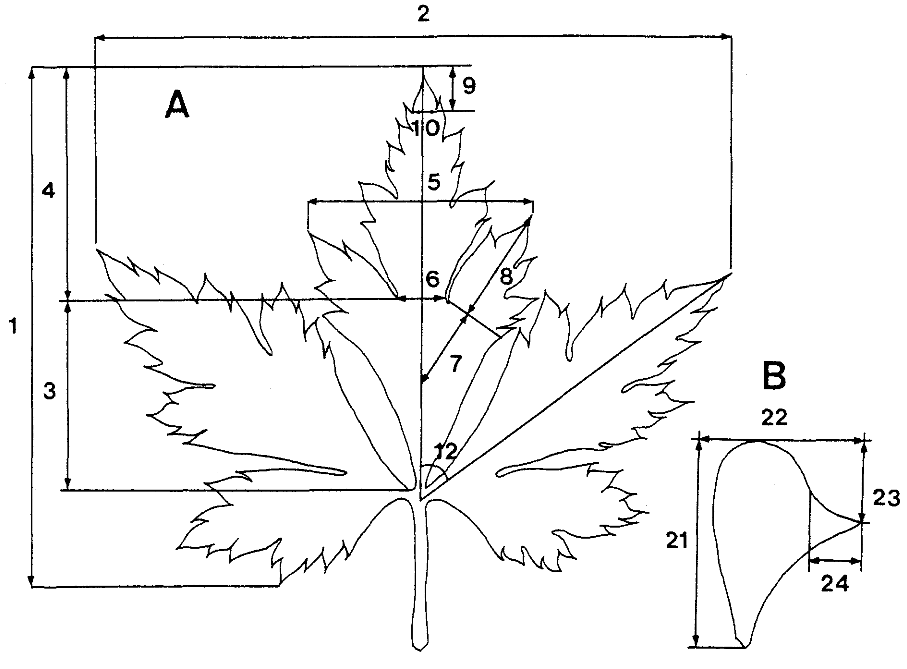

Fig. 2. Diagram showing leaf (A) and floral (B) characters measured for numerical analyses of *Aconitum japonicum* subsp. *napiforme*, *A. jaluense* subsp. *jaluense*, and their putative hybrid. Numbers correspond to character numbers in Table 1.

Table 1. Morphological characters used in numerical analyses of *Aconitum japonicum* subsp. *napiforme*, *A. jaluense* subsp. *jaluense*, and their putative hybrid. See Fig. 2 for further clarification

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Vegetative characters:
1. Leaf length [cm]
2. Leaf width [cm]
3. Distance between the middle lobe base and the primary incision point of the middle lobe [cm]
4. Distance between the middle lobe apex and the primary incision point of the middle lobe [cm]
5. Maximum middle lobe width [cm]
6. Middle lobe width at the primary incision point [cm]
7. Distance between the primary lacinia base and the midvein of the middle lobe [cm]
8. Length of primary lacinia of the middle lobe [cm]
9. Middle lobe apex length [cm]
10. Middle lobe apex width at the base [cm]
11. No. of teeth between the middle lobe apex and the primary incision point [no.]
12. Angle between the middle lobe and the lateral lobe [degree]
13. Character 1/character 2
14. Character 4/(character 3 + character 4)
15. Character 6/character 5
16. Character 5/(character 3 + character 4)
17. (Character 3 + character 4)/petiole length
18. Character 9/character 10
19. Character 8/(character 7 + character 8)
20. Pedicel pubescence: (1) curved hairs only, (2) curved hairs and few spreading glandular hairs, (3) spreading glandular hairs and few curved hairs, (4) spreading glandular hairs only

Floral characters:

21. Helmet (upper sepal) length [cm]
22. Helmet width [cm]
23. Distance between the beak and the helmet apex [cm]
24. Beak length [cm]

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from the first author upon request.

Pedicel hairs of the putative hybrids and the parental taxa were examined with a scanning electron microscope (SEM). Pedicels fixed in FAA were dehydrated in a graded series of acetone concentrations, followed by critical-point-drying in a Polaron Model E3000 with liquid carbon dioxide. Dried samples mounted on aluminum stubs were coated with gold, and examined and photographed using an Akashi Model SR-50A SEM.

Results and Discussion

Pedicel pubescence has been regarded as one of the most useful diagnostic characters in *Aconitum* taxonomy (Nakai 1909, 1914, 1920, 1935, 1937, 1950, 1953, Chung 1957, Tamura and Namba 1959a, 1959b, 1960, Hardin 1964, Park 1974, Wang 1979, Kadota 1981, 1987), and also has been used to infer the origin of intermediate forms in subg. Aconitum (Kadota 1981, 1987).

In all individuals of *A. japonicum* subsp. *napiforme* collected from Cheju Island, which is the type locality of the taxon, pedicels are moderately to densely pubescent with micropapillate curved hairs (Fig. 3A). In contrast, the populations at Mt. Chiri area are composed of plants having only micropapillate curved hairs on pedicels (Fig. 3B) as in typical individuals of *A. japonicum* subsp. *napiforme* and those having a mixture of micropapillate curved hairs and smooth-surfaced unicellular spreading glandular hairs on pedicels (Fig. 3C). The occurrence of these plants having mixed-type pedicel pubescence strongly suggests that hybridization involving *A. japonicum* subsp. *napiforme* and the taxon with smooth-surfaced unicellular spreading glandular hairs on pedicels has occurred in these populations. In Korea, smooth-surfaced unicellular spreading glandular hairs are found on pedicels of *A. jaluense* subsp. *jaluense* (Fig. 3D) and some other species including *A. chiisanense* Nakai, *A. volubile* Pallas, *A. neotortuosum* Nakai, and *A. villosum* Rchb., none of which except *A. chiisanense*, however, occur in Mt. Chiri area. Although *A. chiisanense* is sympatric with *A. japonicum* subsp. *napiforme* in Mt. Chiri area, the former taxon is diploid (2n=16) and the artificial crossing trials of these two taxa completely failed to produce any seeds (Park and Oh unpubl. data). In addition, *A. volubile* and *A. neotortuosum* have twining stems, and *A. villosum* is restricted to the northernmost part of Korea; it is highly unlikely that these taxa are one of the parental taxa of the putative hybrids. Although it is not found in Mt. Chiri area, *A. jaluense* subsp. *jaluense* is relatively common in South Korea and the artificial crossing experiments showed that the genetic barrier between *A. jaluense* subsp. *jaluense* and *A. japonicum* subsp. *napiforme* is incomplete (Park and Oh unpubl. data).

Plants ascribable to *A. japonicum* subsp. *napiforme* but with a mixture of curved hairs and smooth-surfaced unicellular spreading glandular hairs on pedicels were also found in Japan (*A. callianthum* Koidz.) and northeast-

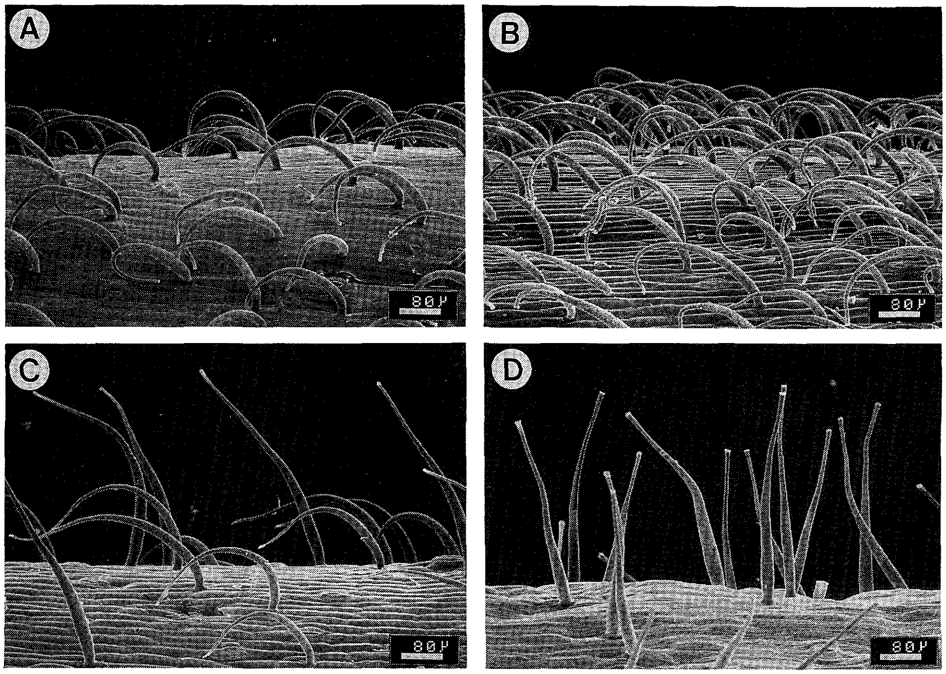

Fig. 3. Pedicel pubescence of *Aconitum japonicum* subsp. *napiforme* (A), individuals of putative hybrid populations (B, C), and *A. jaluense* subsp. *jaluense* (D).

ern China (Kadota 1987); on the basis of pedicel pubescence, Kadota (1987) postulated that they are probably derived from the hybridization between *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense*.

Besides pedicel pubescence, *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* differed strongly in a number of vegetative and floral characters (Figs. 4, 5). T-test (Snedecor and Cochran 1980) showed statistically significant differences at $p<0.001$ between the two taxa for 18 (1–5, 8, 9, 11, 12, 14,

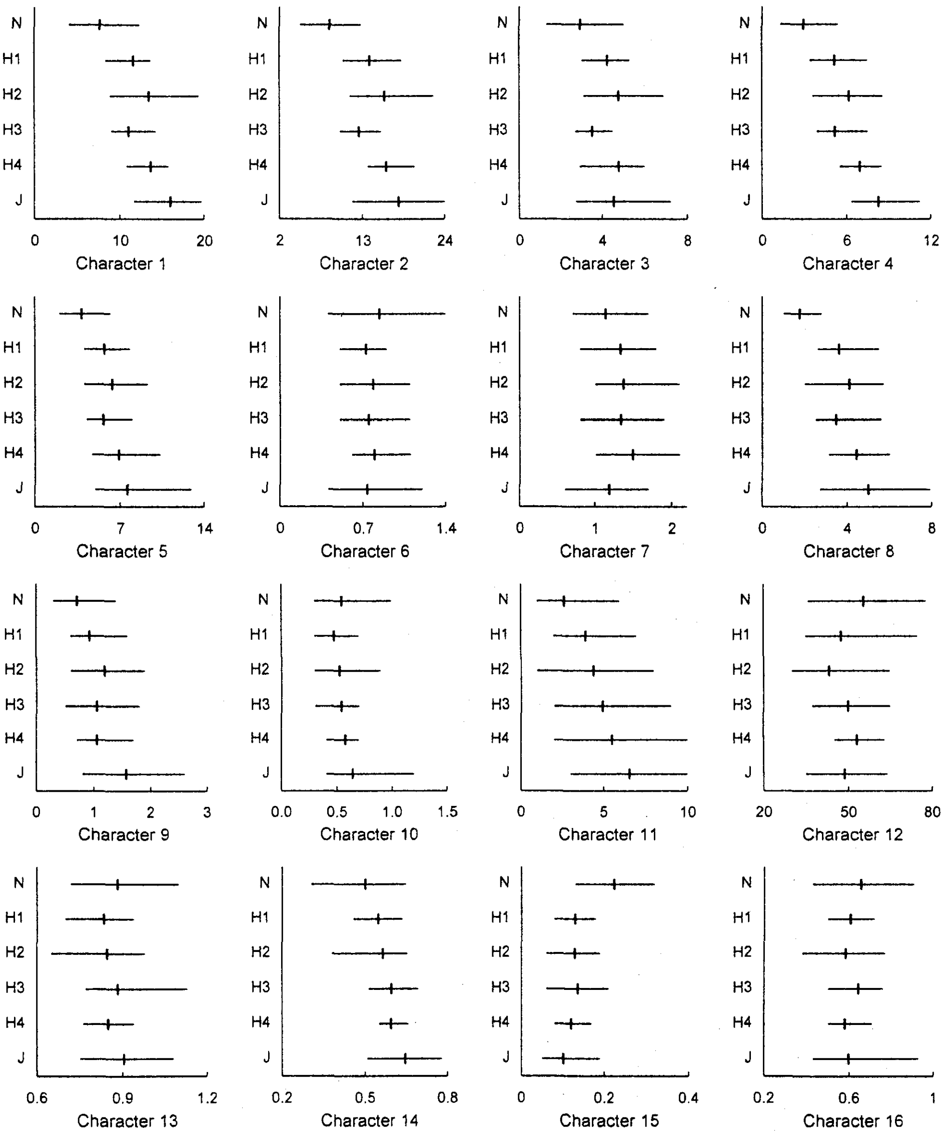

Fig. 4. Ranges and means for morphological characters (characters 1–16) measured from individuals of *Aconitum japonicum* subsp. *napiforme* (N), *A. jaluense* subsp. *jaluense* (J), and their putative hybrid populations (H). Character and hybrid population numbers correspond to those in Table 1 and Fig. 1, respectively.

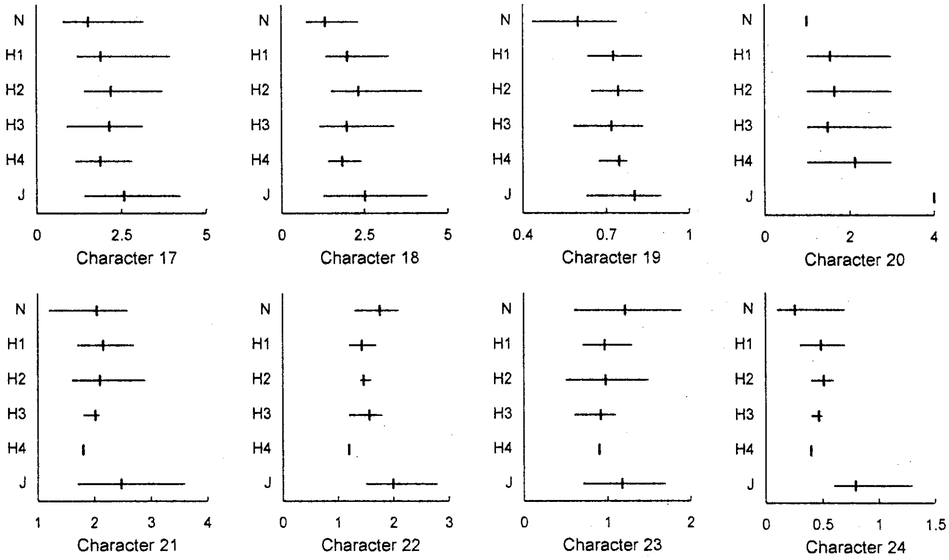

Fig. 5. Ranges and means for morphological characters (characters 17–24) measured from individuals of *Aconitum japonicum* subsp. *napiforme* (N), *A. jaluense* subsp. *jaluense* (J), and their putative hybrid populations (H). Character and hybrid population numbers correspond to those in Table 1 and Fig. 1, respectively.

15, 17–22, 24; parenthetical numbers here and subsequently refer to character numbers in Table 1) of 24 characters measured. Especially noteworthy among the vegetative characters include distance between the middle lobe apex and the primary incision point (4) and length of primary lacinia of the middle lobe (8); the ranges of these characters for the two taxa did not overlap (Fig. 4). Among floral characters, range was almost nonoverlapping for beak length (24) (Fig. 5). In general, flowers of *A. jaluense* subsp. *jaluense* tend to be larger and more hooded with much longer projecting beaks as compared to those of *A. japonicum* subsp. *napiforme*.

Four putative hybrid populations at Mt. Chiri area were, in general, intermediate between *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* for most vegetative and floral characters measured. Of 18 vegetative and floral characters that demonstrated statistically significant differences between *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense*, they were more or less intermediate between these two taxa for all but angle between the middle lobe and the lateral lobe (12), helmet length (21), and helmet width (22) (Figs. 4, 5). In particular, helmet length (21) of the putative hybrid populations were more or less similar to that of *A. japonicum* subsp. *napiforme*, and helmet width (22) was smaller for the putative hybrid populations than for either putative parent (Fig. 5).

Result of the principal components analysis of 179 individuals representing *A. japonicum* subsp. *napiforme*, *A. jaluense* subsp. *jaluense*, and the putative hybrid populations using 20 vegetative characters is provided in Table 2. The first three principal components accounted for 48.2, 13.3, and 7.9 percent of the total variance, respectively (Table 2), and subsequent components contributed less than 6.6 percent each. The first component reflected high positive loadings for leaf length (1), leaf width (2), distance between the middle lobe apex and the primary incision point (4), maximum middle lobe width (5), length of primary lacinia of the middle lobe (8), middle lobe apex length (9), and ratio of primary lacinia length/distance between the primary lacinia apex and the midvein of the middle lobe (19), and a high negative loading for ratio of middle lobe width at the primary incision point/maximum middle lobe width (15). The second component was weighted heavily for middle lobe width at the primary incision point (6) and distance between the primary lacinia base and the midvein of the middle lobe (7). The characters related to the third component were middle lobe apex width at the base (10) and ratio of leaf length/leaf width (13) (Table 2).

The plot of individuals projected on the first two components (Fig. 6) revealed that individuals of *A. japonicum* subsp. *napiforme* are separated clearly from those of *A. jaluense* subsp. *jaluense* by the first component, which is primarily a function of characters related to the leaf size and the degree of leaf incision

Table 2. Loadings of the first three principal components for 20 vegetative characters from the analysis of 179 individuals of *Aconitum japonicum* subsp. *napiforme*, *A. jaluense* subsp. *jaluense*, and their putative hybrid. Character numbers correspond to those in Table 1

Character no.	Component		
	1	2	3
1	0.3027	0.1064	-0.0497
2	0.2961	0.0797	-0.1711
3	0.2204	0.3283	-0.3143
4	0.3131	0.0357	0.0616
5	0.2884	0.0413	0.0232
6	-0.0536	0.5362	0.1723
7	0.0474	0.4717	-0.2083
8	0.3040	-0.0452	-0.0810
9	0.2608	0.0352	0.0890
10	0.1025	0.2683	0.4265
11	0.2178	0.0188	0.1801
12	-0.1116	-0.1044	0.2373
13	0.0190	0.1168	0.4293
14	0.2323	-0.2331	0.3067
15	-0.2667	0.2464	0.1319
16	-0.0769	-0.2103	0.2304
17	0.1851	0.1525	0.2450
18	0.2294	-0.1421	-0.2093
19	0.2789	-0.2376	-0.0251
20	0.2427	-0.0468	0.2318
Eigenvalue	9.6438	2.6656	1.5655
Cumulative % of eigenvalues	48.2	61.5	69.4

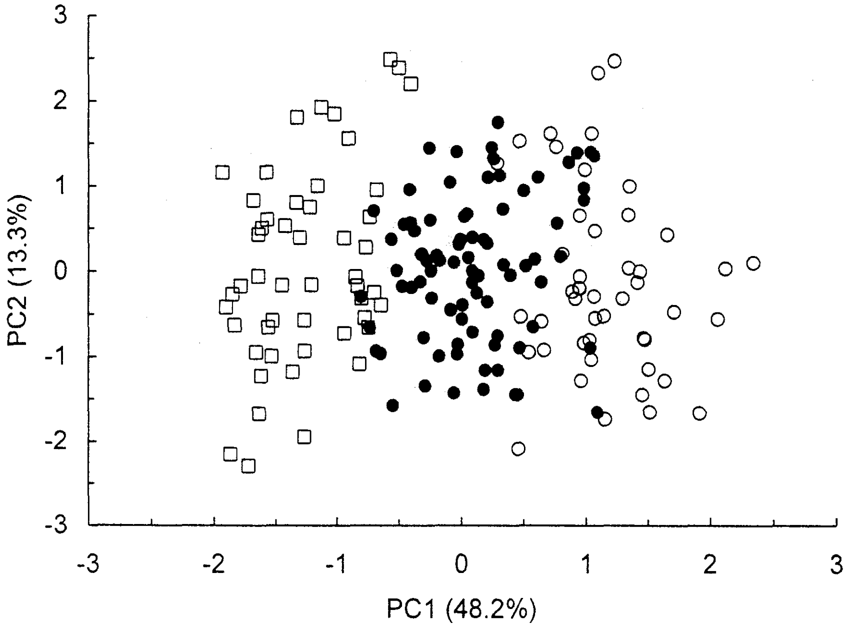

Fig. 6. Principal components analysis of *Aconitum japonicum* subsp. *napiforme*, *A. jaluense* subsp. *jaluense*, and their putative hybrid using 20 vegetative characters (cf. Table 1). Some individuals are hidden due to the same values. Symbols: Open squares = *A. japonicum* subsp. *napiforme*, open circles = *A. jaluense* subsp. *jaluense*, closed circles = putative hybrid.

(Table 2). The relative positions of individuals of these two taxa on the first component (Fig. 6) suggest that plants of *A. jaluense* subsp. *jaluense* tend to have larger leaves with more deeply incised middle lobes and long caudate apices as compared to those of *A. japonicum* subsp. *napiforme*. In *A. japonicum* subsp. *napiforme*, leaves are, in general, shallowly incised and usually have short acuminate apices. Additionally, the number of teeth in middle and lateral lobes is fewer in *A. japonicum* subsp. *napiforme* than in *A. jaluense* subsp. *jaluense* (Fig. 4). However, the second component, which is primarily related to the middle lobe width at the primary incision point failed to separate the two taxa.

Individuals of the putative hybrid populations occupied an intermediate position between those of *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense* with slight overlap on the first component (Fig. 6), indicating that they are somewhat intermediate in leaf size, degree of incision, and apex length. Although leaf size and shape frequently show interpopulational variation in many species of subg. Aconitum due to the habitat differences (Hardin 1964, Kadota 1981, 1987), all eight characters loaded heavily on the first component revealed strong differences between the putative parental taxa, and the ranges for five of these characters showed no or little overlap (Fig. 4). The intermediacy of putative hybrid individuals in the plot, in conjunction with evidence from the pedicel pubescence, strongly suggests that the populations at Mt. Chiri area were originated from hybridization between *A. japonicum* subsp. *napiforme* and *A. jaluense* subsp. *jaluense*.

In addition, the absence of "typical" forms of both *A. japonicum* subsp. *napiforme* and *A.*

jaluense subsp. *jaluense* in the putative hybrid populations as well as in Mt. Chiri area suggests that repeated introgression has probably occurred in these populations. Although some of the individuals in the putative hybrid populations have only micropapillate curved hairs on pedicels as in typical individuals of *A. japonicum* subsp. *napiforme*, they are more or less intermediate in leaf shape and size.

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朴 鍾郁[a]，呉 相勳[b]，林 采恩[a]，徐 榮倍[b]：**韓国智異山における，タンナトリカブトとコウライブシ（キンポウゲ科）の雑種集団**

韓国南部に位置する智異山において，トリカブト属植物（キンポウゲ科）を集団レベルで解析した．この集団にはタンナトリカブトとコウライブシが関与していることが明らかであったため，比較のためにそれぞれ済州島（タンナトリカブト）と韓国南部（コウライブシ）の典型的な集団についても併せて調査を実施した．解析の結果，タンナトリカブトとコウライブシは花梗の有毛性，いくつかの栄養的な器官や花部器官によって明瞭に認識できることが明らかになった．しかし，智異山の集団はこれらの形質で中間性を示した．これらの結果により，智異山の集団は自然雑種形成に由来するものと推定された．さらに，本研究で解析した智異山の4集団ばかりではなく智異山地域全体を通して「典型的な」タンナトリカブトとコウライブシが見られないのは，浸透性交雑が繰り返し起こったことを示唆していると考えられた．

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